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Multispacer Typing Technique for Sequence-Based Typing of *Bartonella quintana*

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Bartonella quintana **is a worldwide fastidious bacterium of the Alphaproteobacteria responsible for bacillary angiomatosis, trench fever, chronic lymphadenopathy, and culture-negative endocarditis. The recent genome sequencing of a *B. quintana* isolate allowed us to propose a genome-wide sequence-based typing method. To ensure sequence discrimination based on highly polymorphic areas, we amplified and sequenced 34 spacers in a large collection of *B. quintana* isolates. Six of these exhibited polymorphisms and allowed the characterization of 4 genotypes. However, the strain variants suggested by the noncoding sequences did not correlate with the results of pulsed-field gel electrophoresis (PFGE), which suggested a higher degree of variability. Modification of the PFGE profile of one isolate after nine subcultures confirmed that rearrangement frequencies are high in this species, making PFGE unreliable for epidemiological purposes. The low extent of sequence heterogeneity in the species suggests a recent emergence of this bacterium as a human pathogen. Direct typing of natural samples allowed the identification of a fifth genotype in the DNA extracted from a human body louse collected in Burundi. We have named the typing technique herein described multispacer typing.**

Bartonella quintana is a fastidious gram-negative rod that infects humans and belongs to the alpha subgroup of the *Proteobacteria* (20, 29, 39). Recent reports suggest that humans are the natural reservoir of *B. quintana* (13) and that the human body louse is the vector (14, 36). Trench fever, occurring in allied and German troops during World War I, was the first disease recognized to be caused by *B. quintana* (24). It was described as a relapsing fever or quintan fever characterized by attacks of fever associated with headaches, skin pain, and dizziness, recurring every 4 to 6 days (24). *B. quintana* is also known to be responsible for endocarditis (9, 15, 34, 42, 43) and bacillary angiomatosis, which occur in both human immunodeficiency virus-infected and immunocompetent patients (23, 29, 36, 37).

More recently, chronic asymptomatic bacteremias and relapsing febrile illness have been reported in homeless populations (5, 13, 32, 44, 45). Up to 14% of bacteremic subjects have been retrieved among homeless people tested in the university hospital in Marseilles, France (5), suggesting an epidemic in this population. It is not known whether the epidemic is due to a single, a few, or many different strains. Some of the patients have persistent bacteremias (duration of up to 78 weeks) (13). The mechanisms for persistence of the infection are currently unknown, but since some patients have a concomitantly high level of antibodies and bacteremia, reinfection by other strains may occur. It was previously demonstrated that specific antibodies did not protect from reinfection by another serotype or genotype in *Bartonella henselae*-infected cats (47). The occurrences of specific pathovars have not been investigated.

The 1.6-Mb genome of *B. quintana* was recently sequenced (2) and found to be a derivate of the larger 1.9-Mb genome of *B. henselae*, with the main difference among the two species residing in the absence of genomic islands in the trench fever agent. The availability of complete genome sequence information for *B. quintana* now allows the rational design of typing methods. To date, only pulsed-field gel electrophoresis (PFGE) has been used for *B. quintana* typing on a limited number of strains (40). This method was applied to seven different isolates of *B. quintana*, and a comparison of the genomic fingerprints showed polymorphisms in DNA restriction patterns, with a specific profile for each isolate (40). Surprisingly, the locations of restriction sites of the cutting endonucleases used for PFGE did not match perfectly with the sizes of fragments obtained with PFGE (unpublished data). This emphasizes the need for more reproducible and convenient typing methods.

Multilocus sequence typing is a new typing method based on the comparison of nucleotide sequences of 450- to 500-bp internal fragments of a number (usually seven) of housekeeping genes (12). For each gene, the different sequences obtained are assigned to alleles; alleles at the seven loci provide an allelic profile, allowing nonambiguous determination of sequence type (12). Multilocus sequence typing was first developed for *Neisseria meningitidis* (12, 27) and *Streptococcus pneumoniae* (11, 12). It has by now been described for several human pathogens, including *B. henselae* (19), enabling the identification of seven sequences types among a total of 37 human and feline isolates.

The aim of this study was to develop a simple and reproducible typing method for *B. quintana* based on highly polymorphic sequence regions. Since spacers (intergenic and pseudogene sequences, also called junk DNA) are more variable than gene sequences, we have here examined a molecular typing method based on the sequences of such noncoding zones rather than of housekeeping genes. This approach was highly successful and we have named the technique MST, which stands for multispacer typing (10).

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TABLE 1. List of *B. quintana* isolates tested in this work and repartitioned according to genotype for spacers 471 and 894

Isolate	Yr	Sample source	Disease	Origin	Sequence type for spacer:	
					471	894
1084 SDF 2000	2000	Blood culture	Bacteremia[a]	Marseilles, France	ND[b]	2
22	2001	Blood culture	Bacteremia	Marseilles, France	ND	2
41 647	2000	Blood culture	Bacteremia	Marseilles, France	2	2
44 235	2000	Blood culture	Bacteremia	Marseilles, France	2	2
57	2000	Louse		Marseilles, France	ND	2
Fuller	1948	Blood culture	Trench fever	Yugoslavia	3	3
Grenoble	1994	Strain	Bacillary angiomatosis	Grenoble, France	2	2
Marseille	1993	Blood culture	Lymphadenopathy	Marseilles, France	2	2
Oklahoma	1992	Blood culture	Bacillary angiomatosis	Oklahoma	2	2
Paris	1993	Blood culture	Endocarditis	Paris, France	ND	2
SH-perm		Louse		Russia	2	2
Toulouse	1992	Strain	Bacillary angiomatosis	Toulouse, France	3	3
UR.BQ.M.NHP.140	2001	Louse		Marseilles, France	ND	2
UR.BQ.M.NHP.145A	2001	Louse		Marseilles, France	ND	1
UR.BQ.M.TD.148	2001	Blood culture	Bacteremia	Marseilles, France	ND	1
UR.BQ.M.TF.141	2001	Blood culture	Bacteremia	Marseilles, France	1	1
UR.BQ.M.TF.141C	2001	Blood culture	Bacteremia	Marseilles, France	1	1
UR.BQ.M.64	1999	Lymph node	Lymphadenopathy	Marseilles, France	ND	2
UR.BQ.M.AS.13	1997	Blood culture	Bacteremia	Marseilles, France	2	2
UR.BQ.M.AS.15	1997	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.IE.48	1999	Blood culture	Endocarditis	Marseilles, France	ND	2
UR.BQ.M.L.Y.1.5	1994	Bone marrow	Lymphadenopathy	Marseilles, France	ND	2
UR.BQ.M.NHP.108	2000	Louse		Marseilles, France	ND	2
UR.BQ.M.NHP.147C	2001	Louse		Marseilles, France	ND	2
UR.BQ.M.NHP.151E	2001	Louse		Marseilles, France	ND	1
UR.BQ.M.NHP.158B	2001	Louse		Marseilles, France	ND	2
UR.BQ.M.NHP.91	2000	Louse		Marseilles, France	1	1
UR.BQ.M.TF.101	2000	Blood culture	Bacteremia	Marseilles, France	2	2
UR.BQ.M.TF.105	2000	Blood culture	Bacteremia	Marseilles, France	1	1
UR.BQ.M.TF.122	2000	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.131	2000	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.138	2001	Blood culture	Bacteremia	Marseilles, France	ND	1
UR.BQ.M.TF.139	2001	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.141C	2001	Blood culture	Bacteremia	Marseilles, France	1	1
UR.BQ.M.TF.142	2001	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.142B	2001	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.146	2001	Blood culture	Bacteremia	Marseilles, France	ND	ND
UR.BQ.M.TF.146B	2001	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.146D	2001	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.146E	2001	Blood culture	Bacteremia	Marseilles, France	2	2
UR.BQ.M.TF.148B	2001	Blood culture	Bacteremia	Marseilles, France	1	1
UR.BQ.M.TF.149	2001	Blood culture	Bacteremia	Marseilles, France	1	1
UR.BQ.M.TF.150	2001	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.152	2001	Blood culture	Bacteremia	Marseilles, France	ND	1
UR.BQ.M.TF.153	2001	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.16	1997	Blood culture	Bacteremia	Marseilles, France	2	2
UR.BQ.M.TF.20	1997	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.201B	2002	Blood culture	Bacteremia	Marseilles, France	ND	ND
UR.BQ.M.TF.202A	2002	Blood culture	Bacteremia	Marseilles, France	ND	ND
UR.BQ.M.TF.202F	2002	Blood culture	Bacteremia	Marseilles, France	ND	ND
UR.BQ.M.TF.21	1997	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.22	1998	Blood culture	Bacteremia	Marseilles, France	ND	1
UR.BQ.M.TF.24	1998	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.36	1998	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.38	1999	Blood culture	Bacteremia	Marseilles, France	ND	1
UR.BQ.M.TF.39	1999	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.41	1998	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.42	1998	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.43	1998	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.44	1998	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.47	1999	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.60	1999	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.61	1999	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.65	1999	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.66	1999	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.85	2000	Blood culture	Bacteremia	Marseilles, France	ND	2
UR.BQ.M.TF.88	2000	Blood culture	Bacteremia	Marseilles, France	2	2
UR.BQ.P.BA.A.7	1995	Strain	Bacillary angiomatosis	Paris, France	2	2
UR.BQ.P.IE.10	1996	Blood culture	Endocarditis	Paris, France	ND	2
UR.BQ.P.IE.62	1999	Cardiac valve	Endocarditis	Paris, France	ND	2
UR.BQ.P.TF.162	2001	Blood culture	Bacteremia	Paris, France	ND	2

[a] All cases of bacteremia were found in homeless people.
[b] ND, not done.

TABLE 2. List of primers used for amplification and sequencing of spacers used in this study

Spacer	Size (bp)[a]	Forward primer	Reverse primer
81	639	TCTTGAGTGGTGGAAGCAA	TGACCACAATAATCTAATTC
85	693	AATCTTCTGGCTTTTAATGAAATG	ATTCAATGACATATCAAAATGATC
91	702	GCAATTATTAAGGCCACAGT	ACCGCAATGAAAGCTCCATA
112	793	TGTTGCTCCTGCTGCCAAAA	GCTCAATGTGTTCTTTATCA
128	1,077	GCTGTAATTGGTGAAGTTCT	TCTTCGCAGGACTATAAATA
129	1,136	AGTGATAGGCAGCAATCTTG	TCTGATATTCATGCTTATGATACG
136	654	ATTGTAAGCGGAGTTCTCAAAATC	TTACATTGGTGGTCAGCATCTTTC
144	1,158	CAAGTGGCTAAGATTATGTT	TGACTTACAGATTCTATGCC
170	1,375	CAAAATTGGCGAGCTTCAC	ATAACCTGGCGTTTGCTCAT
178	908	AAGCCTTTTGCGATTCTACCC	TCAGCCCAATCAATGCGAAG
192	726	TTCTCGTTATCCTCTCGTATG	CCTGCAATGCTGAATTTTGG
219	702	GTTTTTAGTACGCCTCCCAC	CTCTATCCAAAGCACAAACT
253	716	TTTATTCCCGGTAACATGCC	AGAAAGCGGCGCATATCGTT
303	746	AACAGCATCAACGAACAAGC	GGAGTTGTCTAAAGGAAGGA
322	1,158	AGATACTCCTTTCGTGCTGC	TTTTACGTGAGGCGGGAAAG
336	740	GCTAAGAAAGAAAGCGAAGC	ATGCTAACCCACTAAAACGG
339	1,501	CCGCACTGAGAATTTTAGAG	GGCGAAAGGGCATCATAATC
352	1,733	CGAAACATCTATCGCCAAAC	GAGAAAAAGCACTGGCTATT
355	1,194	TATATTCAGCGCGGTAATCG	CTTAGGAAGAGTGTAGATGA
385	999	AACACATCGCAATGAATCCG	GCAACATCTTCAGCAAGACG
405	676	TTTCTATCTGATAATGACTCAAACC	ATTCCCGCTACGAGAAAGAAAAC
437	848	GATAAAGGAAGCATTCATCG	ATCGTCATCAAGGCAAAAAC
438	837	AGTAAAGCTACTCTAAAGCC	CCTTCCCATTGTTTAATCAG
470	1,468	TAACATCTCTTGCGTTAAGC	TGAGGTCGATATTTACGAGC
471	869	AGCCTTACAGGCAAGAACAAATC	ATGATAGTAAAAGCAATGATAAACC
472	826	AGGATAGTGCATTATTTTAGC	TTTATGACGAATGAGCGCAA
507	1,419	TGATCCAGCAAGTGCTTCAG	GTTCGTCTACGAGCGTTGTT
531	948	GATATTATGAAAAACCATCAG	AAGATAAATGGTGCGAGCAC
581	740	ACCAGAAGCAAGTGACGTTC	GATATGAGTGCTTCTGTTGT
593	690	ATTTTTCTTTTGAACAAATGC	GCGGCATCATAGTCATTACC
597	827	GCCGCTTATTGTTGTCGATC	CGGCGTCTCAGTATAGAATT
598	821	CCATAAGCAGCAAGTGCGAT	AGCAATGATGGCACTCTTGC
894	683	CGGTTTGTAACGCTCTCAATGGA	TCAACGTAATCGTTCTCTGTGTC
895	809	ATATGCAAAAAGGCAAATGACCTG	TTAGGAATATCAACCAAAACTGATC

[a] Sizes refer to estimated sizes of PCR products according to the genome sequence of the *B. quintana* Toulouse strain (2).

MATERIALS AND METHODS

***B. quintana* isolates and growth conditions.** The 71 isolates analyzed in the present study are listed in Table 1. PFGE analysis of the Toulouse, Fuller, Grenoble, Oklahoma, SH-perm, Marseille, and Paris strains have already been described elsewhere (40). Methods used for primary isolation of *B. quintana* from clinical samples have been described elsewhere (26). Subcultures were performed on blood agar (BioMerieux, Marcy l'Etoile, France) at 37°C in 5% CO_2 (Genbag CO_2 system; BioMerieux). Four agar plates were obtained for each isolate. Bacteria were harvested and suspended in 500 μl of sterile water after 3 to 5 days of culture.

Amplification, sequencing, and PFGE conditions. A total of 1,427 open reading frames were annotated in the complete genome of *B. quintana* (Toulouse strain) (2). To propose a typing method based on the sequencing of a fragment amplified from a single PCR run, we retained for analyses only noncoding sequences of 500 to 1,500 bp. We analyzed 178 such sequences, of which 34 were selected for amplification and sequencing with primers (Table 2) designed by using the primer design tool (CyberGene AB).

DNA was extracted by using the Chelex procedure (7). PCR conditions for all amplification reactions were as follows: initial denaturation at 94°C for 3 min, followed by 44 cycles (94°C for 30 s, 50 to 61°C [according to the melting temperatures of the primers] for 30 s, and 68°C for 90 s), and final extension at 68°C for 7 min. Reactions were performed in 25-μl volumes with buffers and Elongase from Invitrogen Life Technologies (Cergy Pontoise, France). PCR products were visualized under UV illumination after electrophoresis migration on a 1% agarose gel stained with ethidium bromide. PCR products were purified by using the MultiScreen PCR filter plate (Millipore, Saint-Quentin en Yvelines, France) as recommended by the manufacturer.

The PCR products were sequenced in both directions by using the D-rhodamine terminator cycle sequencing ready reaction kit (PerkinElmer, Coignières, France) according to the manufacturer's recommendations. Sequencing products were resolved in an Applied Biosystems automatic sequencer model 3100 (PerkinElmer). As a negative control of each amplification reaction, we used sterile water processed as described above.

PFGE after BstZI restriction endonuclease digestion of *B. quintana* strains was performed as previously described (40).

Sequence analysis and data deposition. The nucleotide sequences were edited with the Autoassembler package (PerkinElmer). Multiple alignment of sequences was carried out by using the CLUSTALW webware (http://pbil.ibcp.fr) (46). The sequences of the different genotypes for the six discriminating spacers have been deposited in the EMBL/GenBank databases and given accession numbers, as shown in Table 3.

RESULTS

We have here examined the variability of 71 *B. quintana* isolates (Table 1) based on noncoding sequences as inferred from the complete genome sequence of *B. quintana* (2). Since the aim was to develop a typing method based on single PCR runs, the first selection criteria were that the sizes of the noncoding sequences should range from 500 to 1,500 bp. Of 178 sequences examined, primer pairs were designed for 34 noncoding segments. These were subjected to several rounds of screenings and selections with the aim of identifying the most useful set of sequences for genotyping purposes (Table 2). Both gene and pseudogene sequences were used as anchors for primer design. Three of the selected sequences contain internal tRNA genes (128, 129, and 339) and another two pseudogenes (144 and 178) in between the flanking gene sequences. Finally, two re-

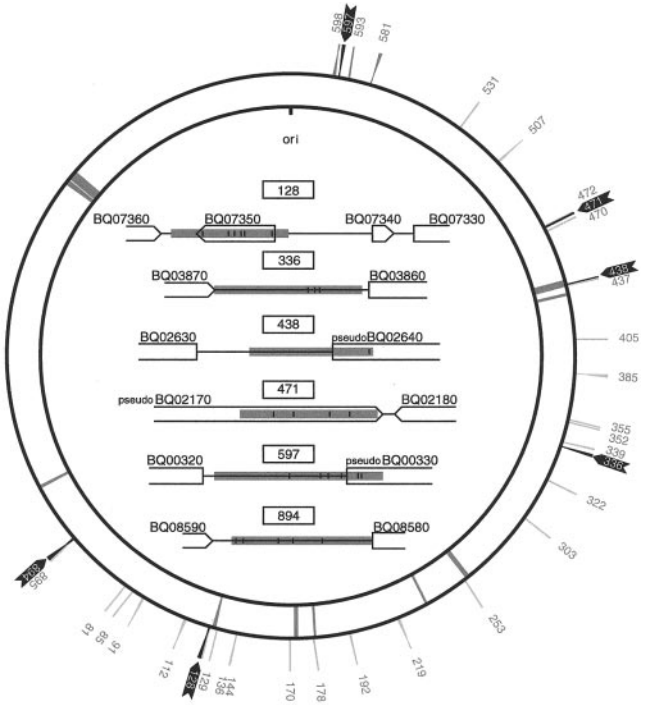

FIG. 1. Position and organization of noncoding sequence segments with polymorphisms in *B. quintana* strains. The outer circle shows the positions of the noncoding sequences analyzed by PCR. Polymorphic sequences are highlighted with black arrows, and sequences found only in one variant are shown in grey. The inner circle indicates the location of remnants of the prophage and genomic islands present in the *B. henselae* genome (2). The organization of the junk sequences with polymorphisms is displayed inside the two circles. Grey areas show the locations of the sequenced regions. The positions of the polymorphic sites, including both nucleotide substitutions (bars) and deletions (triangles), are shown within the sequenced regions.

gions contain internal short open reading frames (128 and 352) putatively coding for proteins of unknown function. The position of the 34 PCR-amplified segments in the genome of the Toulouse strain of *B. quintana* are shown in Fig. 1.

Screening for spacers displaying sequence variants. We hypothesized that the most polymorphic sequences may be those under no or weak selective constraints, i.e., sequences with the lowest similarity to their homologous sequences in *B. henselae*. For 10 segments, less than 10% of the sequence showed ≥80% similarity with its counterpart in *B. henselae*. These were selected for a first round of PCR amplifications in the *B. quintana* isolates Toulouse, Fuller, and Oklahoma, i.e., strains that are well discriminated by PFGE (40). Segment 471, which is flanked on one side by the pseudogene BQ02170 and on the other by the gene BQ02180, and segment 894, which consists of a spacer flanked by the genes BQ08590 and BQ08580, allowed the differentiation of the Toulouse and Fuller strains from the Oklahoma strain (Fig. 1). In total, 4 and 192 nucleotide differences were revealed for segments 471 and 894, respectively. To examine whether additional sequence variants exist, we sequenced region 471 in 18 strains and region 894 in 64 *B. quintana* isolates, and this allowed the determination of a third genotype. Those three different variants were observed for each of the two segments and were always associated. Following these results, we amplified and sequenced all of the remaining segments for the *B. quintana* strains UR.BQ.M.TF.141, Oklahoma, and Fuller. Among these, we identified four segments (128, 336, 438, and 597) that displayed sequence variability among the three isolates (Fig. 1). With a total of 17, 6, 10, and 9 nucleotide differences, respectively, these were retained as four additional, putative targets for MST.

Variability in noncoding sequences of selected isolates. To examine the extent of variability in a broader set of strains, we sequenced spacers 128, 336, 438, 597, and 894 in 15 isolates (Table 4). These strains were selected on the basis of differences in PFGE profiles (40), clinical manifestations (homeless people with trench fever, endocarditis, and bacillary angiomatosis), and geographic origins. In this broader analysis, spacer 128 revealed three sequence types, consisting of single-nucleotide polymorphisms (SNPs) at five positions and one deletion of 12 bp. Spacer 336 distinguished the Fuller strain from all other strains based on SNPs at two positions and a deletion of 4 bp. Two sequence types were associated with spacer 438; these differed by a 10-bp deletion in the pseudogene sequence of BQ02640. Spacer 597 classified the isolates into three types on the basis of SNPs at 4 positions, one deletion of 1 bp, and a deletion of 5 bp. Finally, the sequence variants of spacer 894 included two SNPs, one single-nucleotide deletion, and a large deletion of 189 bp. Spacers 128, 597, and 894 were redundant in the sense that they yielded identical classification results. Taken together, the data suggest the presence of four genotypic variants among the 15 isolates (Table 4).

Direct MST of *B. quintana* from louse and human samples. The use of MST was tested on recently isolated samples that previously tested positive for *B. quintana* by PCR methods described elsewhere (41). These included five lice collected from homeless people in Marseilles (France), four lice collected in Burundi, and one positive cardiac valve sent to our laboratory after cardiac valve surgery in a man from London (England) presenting with endocarditis (Table 5). With the aid of segments 336 and 438, the 10 samples were classified into two types for each segment. Interestingly, four different sequence variants were identified for segment 894, including the three previously observed plus a new sequence variant present in one of the four lice isolated in Burundi. Thus, four different genotypes were identified among the nine louse samples. These encompass all of the previously observed genotypes, with the exception of the sequence variant in the bacillary angiomatosis patient from Toulouse plus a novel, fifth genotype (Table 5).

Patterns of nucleotide changes and insertion/deletion mutations. Among the noncoding sequences examined here, we

TABLE 3. GenBank accession numbers of sequences of different genotypes for the six discriminating spacers

Spacer	Accession no. for genotype:			
	1	2	3	4
128	AY660702	AY660703	AY660704	
336	AY660705	AY660706		
438	AY660707	AY660708		
471	AY660716	AY660717	AY660718	
597	AY660709	AY660710	AY660711	
894	AY660712	AY660713	AY660714	AY660715

TABLE 4. Repartitioning of 15 selected *B. quintana* isolates according to results of PFGE and MST

Isolate	Yr	Sample source	Pathology	Origin	PFGE type	Sequence type for spacer:					Genotype
						128	336	438	597	894	
UR.BQ.M.TF.141	2001	Blood culture	Bacteremia[a]	Marseilles, France	1	1	1	1	1	1	1
UR.BQ.M.TD.148	2001	Blood culture	Bacteremia	Marseilles, France	1	1	1	1	1	1	1
UR.BQ.M.NHP.145A	2001	Louse		Marseilles, France	1	1	1	1	1	1	1
Marseille	1993	Blood culture	Lymphadenopathy	Marseilles, France	5	2	1	2	2	2	2
UR.BQ.M.LY.I.5	1994	Bone marrow	Lymphadenopathy	Marseilles, France	2	2	1	2	2	2	2
UR.BQ.P.BA.A.7	1995	Blood culture	Bacillary angiomatosis	Paris, France	4	2	1	2	2	2	2
Paris	1993	Blood culture	Endocarditis	Paris, France	5	2	1	2	2	2	2
UR.BQ.P.IE.62	1999	Cardiac valve	Endocarditis	Paris, France	4	2	1	2	2	2	2
UR.BQ.M.TF.146B	2001	Blood culture	Bacteremia	Marseilles, France	1	2	1	2	2	2	2
UR.BQ.M.NHP.140	2001	Louse		Marseilles, France	4	2	1	2	2	2	2
Oklahoma	1992	Blood culture	Bacillary angiomatosis	Oklahoma	2	2	1	2	2	2	2
SH-perm	Unknown	Unknown	Trench fever	Russia	3	2	1	2	2	2	2
Grenoble	1994	Blood culture	Bacillary angiomatosis	Grenoble, France	2	2	1	2	2	2	2
Toulouse	1992	Blood culture	Bacillary angiomatosis	Toulouse, France	4	3	1	2	3	3	3
Fuller	1948	Blood culture	Trench fever	Yugoslavia	3	3	2	2	3	3	4

[a] All cases of bacteremia were found in homeless people.

identified 12 SNPs and seven insertions/deletions affecting a total of 222 nucleotides. All but one of the single-base substitutions represents GC-AT changes, and only one is an A-T mutation. The insertion/deletion mutations range from single-base deletions to a large deletion of 189 nucleotides. The two single-base deletions are in homopolymeric tracts of 4 to 6 A's or T's in a row. Three indels of sizes 4 to 10 bp are direct repeats of 4 to 10 nucleotides, and the large deletion of 189 nucleotides is flanked by a repeated sequence of 12 bp, in accordance with previous studies which have suggested a role for repeated sequences in the generation of deletion mutations (15).

Lack of correlation between sequence and structure. After digestion with BstZI restriction endonuclease, we found five different patterns among the 15 selected *B. quintana* isolates (Fig. 2; Table 3). The isolates UR.BQ.M.TF.141, UR.BQ.M.TD.148, UR.BQ.M.TF.146B, and UR.BQ.M.NHP.145A were similar to each other and were classified into PFGE type 1 (Fig. 2, domain 1). The UR.BQ.M.LY.I.5 isolate and the Oklahoma and Grenoble strains demonstrated similar patterns (PFGE type 2) (Fig. 2, domain 2), as did the SH-perm and Fuller isolates (PFGE type 3) (Fig. 1, domain 2). Likewise, strains UR.BQ.P.BA.A.7, Toulouse, UR.BQ.P.IE.62, and UR.BQ.M.NHP.140 were similar to each other and categorized into PFGE type 4 (Fig. 2, domain 2). Finally, the Marseille and Paris isolates exhibited identical profiles, referred to here as PFGE type 5 (Fig. 2, domain 3). Curiously, no correlation was retrieved between noncoding sequence types and PFGE types (Table 3). For example, for the BQ.M.TF.146D isolate, the PFGE pattern demonstrated differences before and after subcultures, whereas the MST profile remained the same (Fig. 3).

DISCUSSION

This study has revealed a surprisingly low level of sequence polymorphisms in the noncoding DNA of the examined *B. quintana* strains. Initially, we speculated that intergenic sequences including pseudogenes not under selective pressure should be a likely source of sequence variability among strains of the same species. Indeed, one of the most widely used intergenic spacers, the 16S-23S rRNA spacer region, exhibits extensive variability in many bacterial species, including *Streptococcus* sp. (18), *Tropheryma whipplei* (28), and mycobacteria (30). However, the low frequency of polymorphisms in *B. quintana* is in agreement with a previous study in which 16S-23S rRNA spacer sequencing allowed the identification of a specific sequence for each of the tested *B. henselae* isolates, while the *B. quintana* isolates fell into only two different groups (40).

Moreover, recent data obtained from complete genome sequencing of the two species suggest that *B. quintana* is a subset of *B. henselae*, with an evolutionary scenario that involves losses and rearrangements in the *B. quintana* genome (2). Such losses of sequences, associated with rearrangements, have been described previously for parasitic bacteria (16). For example, the genome of *Rickettsia prowazekii* (3), another louse-borne pathogen, is a subset of the genome of a close relative, *Rickettsia conorii* (31). Because the emergence of the human body louse may be no older than 100,000 years (21), its strict coevolving pathogens, such as *R. prowazekii* and *B. quintana*, are likely to represent recently evolved species. A recent adaptation of *B. quintana* to its unique human host and louse vector would provide an explanation for the low level of sequence variability in the genomes of the natural isolates of this species.

Another curious finding was the more extensive variation of the PFGE profiles than of the MST results. Our hypothesis is that this variability is due to frequent genome rearrangements in *B. quintana*, a scenario that is supported by the modification

TABLE 5. Results of MST performed on samples

Isolate	Yr	Sample source	Origin	Sequence type for spacer:			Genotype
				336	438	894	
15469	2002	Louse	Marseilles, France	1	1	1	1
16763	2002	Louse	Marseilles, France	1	2	2	2
21007	2003	Louse	Marseilles, France	1	2	2	2
21034	2003	Louse	Marseilles, France	1	2	2	2
16311	2002	Louse	Marseilles, France	1	2	2	2
12733	2001	Louse	Burundi	2	2	3	4
12735	2001	Louse	Burundi	2	2	3	4
12737	2001	Louse	Burundi	2	2	3	4
12739	2001	Louse	Burundi	1	1	4	5
10529	2000	Cardiac valve	London, England	1	2	2	2

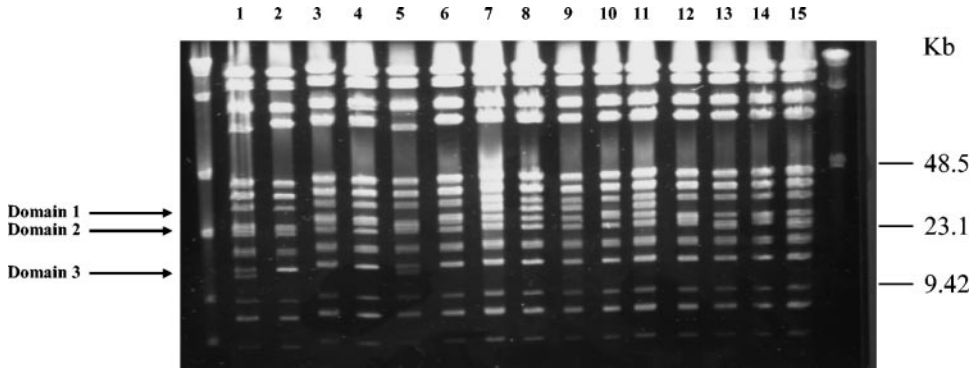

FIG. 2. PFGE of BstZI restriction fragments of *B. quintana* DNAs. Lane 1, Marseille strain; lane 2, UR.BQ.M.LY.I.5 strain; lane 3, UR.BQ.P.BA.A.7 strain; lane 4, Toulouse strain; lane 5, Paris strain; lane 6, UR.BQ.P.IE.62 strain; lane 7, UR.BQ.M.TF.141 strain; lane 8, UR.BQ.M.TD.148 strain; lane 9, UR.BQ.M.TF.146B strain; lane 10, UR BQ.M.NHP.140 strain; lane 11, UR.BQ.M NHP.145A strain; lane 12, Oklahoma strain; lane 13, SH-perm strain; lane 14, Grenoble strain; lane 15, Fuller strain.

of the PFGE profile in one strain after nine subcultures while its MST profile remained the same. Such rearrangements have already been demonstrated with *Yersinia pestis*, in which it appeared as if different colonies obtained from the same strain displayed different PFGE patterns (17). It has recently been demonstrated that long repeated sequences are located in the genomic islands of the *B. henselae* genome and that the constituting remnants of these islands are associated with rearrangements in *B. quintana* (2). Genome rearrangements triggered by repeats have already been described for other bacteria, including *T. whipplei* (4, 35), *Y. pestis* (8, 22, 33), *Mycoplasma* spp. (38), and *Anaplasma* (1, 6). Gene degradations, gene duplications, and genome rearrangements are the main forces that allow evolution and niche adaptation of intracellular pathogens, as acquisition of genes from their eukaryotic host appear insignificant (25, 31, 35). Taken together, the data suggest that PFGE is not a convenient tool for molecular typing of bacteria with extensive genome rearrangements, like that of *B. quintana*. Based upon our data, DNA rearrangement does not compromise the genotyping utility of MST, as MST profiles were not found to be modified after several subcultures.

Finally, using MST based on five noncoding segments allowed us to identify four genotypes of *B. quintana*. This study did not, however, allow the association between a given genotype and the disease associated with *B. quintana* infection. We found that genotype 1 is observed in bacteremic homeless people only (three strains), that, in France, genotypes 1 (three strains), 2 (eight strains), and 3 (one strain) may be observed, that genotype 2 was found for the Oklahoma isolate, the sole New World strain tested, and that the oldest isolate (Fuller)

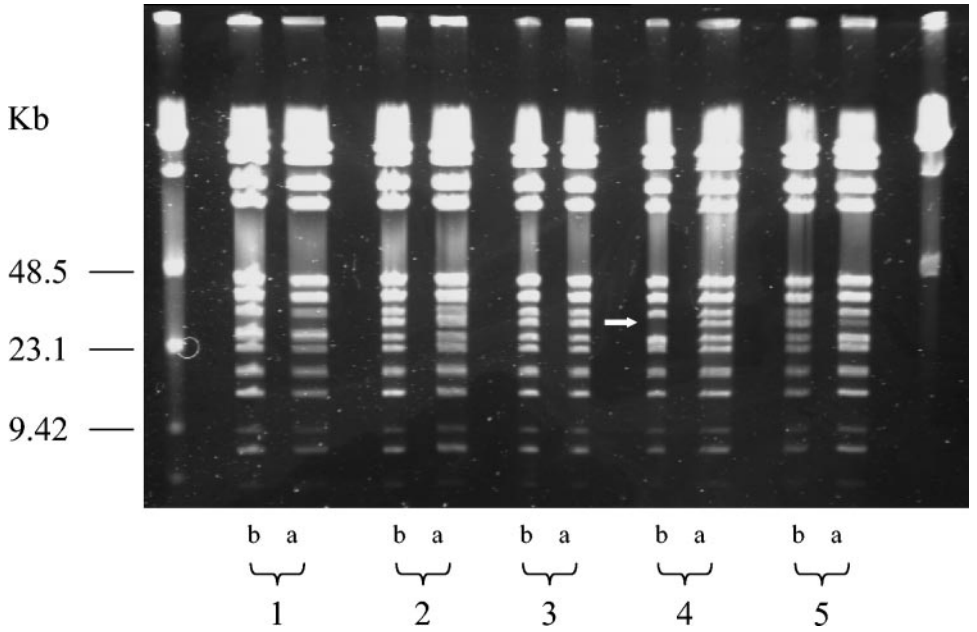

FIG. 3. PFGE of BstZI restriction fragments of five randomly selected *B. quintana* DNAs of strains after nine subcultures (a) compared with the pattern of the initial strain (b). Lane 1, UR.BQ.M.TF.202.A isolate; lane 2, UR.BQ.M.TF.202.F isolate; lane 3, UR.BQ.M.TF.146 isolate; lane 4, UR.BQ.M.TF.146D isolate; lane 5, UR.BQ.M.TF.201.B isolate. The white arrow shows a variation in the PFGE profile of the BQ.M.TF.146D isolate before and after subcultures.

was of the original genotype, genotype 4. Unfortunately, a full analysis of the results is hampered by the overrepresentation of strains from France and homeless people. This is mostly due the fastidiousness of *B. quintana*, especially in patients that are not bacteremic homeless people (26). MST needs to be applied to large collections of *B. quintana* isolates, so we may know whether it will provide relevant clinical, microbiological, and epidemiological data.

Diagnosis of bacillary angiomatosis and endocarditis are mostly based on the results of serology and/or results of PCR amplification of tissue samples (26), encouraging us to directly test spacer typing on natural samples. Interestingly, four different genotypes were recovered from clinical samples (lice and cardiac valves) by using MST. This allowed the characterization of an additional genotype (genotype 5). Two of the genotypes (1 and 2) were observed in lice collected from homeless people in Marseilles (France), and two others (4 and 5) were observed in lice collected in Burundi. The five genotypes depend on the determination of the sequences of only two spacers (336 and 894). With the exception of the Fuller strain, which is an old isolate, it can be concluded that the *B. quintana* genotypes now circulating in Africa are different from those observed in Europe. MST likely represents a novel approach for typing of bacteria with low levels of polymorphism and circumvents problems associated with recurrent genome rearrangements. MST allowed the definition of five genotypes within *B. quintana* species and will now be applied to large collections of isolates and samples from different areas in the world to investigate the geographical distribution of genotypes.

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